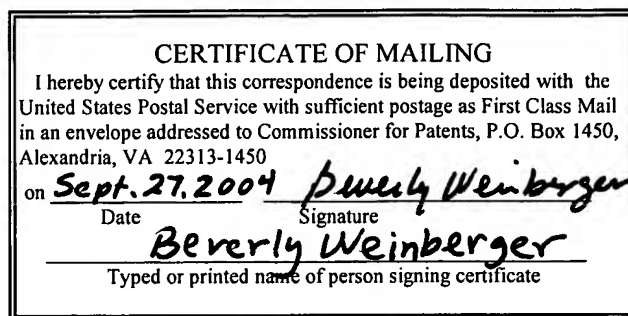


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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Mustapha Abdelouahed and John W. Lawler
Application No.: 10/084,832 Group: 1641
Filed: February 27, 2002 Examiner: D. Davis
Confirmation No.: 5718
For: Diagnostic Assay for Type 2 Heparin-Induced Thrombocytopenia



APPEAL BRIEF

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Appeal Brief is submitted pursuant to the Notice of Appeal received in the U.S. Patent and Trademark Office on June 28, 2004, and in support of the appeal from the final rejections set forth in the Office Action mailed on December 30, 2003. The fee for filing a brief in support of an appeal is enclosed. A Petition for Extension of Time and the appropriate fee are being filed concurrently.

I. REAL PARTIES IN INTEREST

The real parties in interest are Mustapha Abdelouahed and John W. Lawler, who own the entire right, title and interest in the subject application.

II. RELATED APPEALS AND INTERFERENCES

Appellants and the undersigned Attorney are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-7 and 34-37 have been finally rejected, and these claims appear in the Appendix of this Brief. Claims 1 and 2 were amended in the Amendment mailed to the United States Patent and Trademark Office on 19 September 2003. Claim 37 was added in a Preliminary Amendment mailed to the United States Patent and Trademark Office on 30 April 2003. Claims 3-7 and 34-36 are as originally filed. Claims 8-33 have been withdrawn. No claims have been canceled.

IV. STATUS OF AMENDMENTS

No Amendment After Final Rejection has been filed. The most recent Amendment was mailed to the United States Patent and Trademark Office on 19 September 2003 and was entered on 23 September 2003.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The invention is a ternary complex formed from heparin, platelet factor 4 and thrombospondin-1, which, according to the specification, is an immunogen that is bound by antibodies present in the blood of patients with heparin-induced thrombocytopenia. The invention is also a kit comprising the complex.

Support for Claims 1-7 can be found throughout the specification, but especially in the experimental demonstration of the formation of the ternary complexes on page 27, line 18 to page 28, line 16. Support for Claims 34-37 is found on page 7, line 24 to page 8, line 11, on page 23, lines 3-20, and in the original Claims 34-36, for example.

VI. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

Three grounds of rejection raised by the Examiner during prosecution are to be reviewed on appeal. They are:

- (1) anticipation under 35 U.S.C. § 102(b) by Amiral (US 5,466,582) as evidenced by Thorpe *et al.* (US 6,312,694), applied to Claims 1-7;
- (2) anticipation under 35 U.S.C. § 102(b) by Gogstad *et al.* (*British Journal of Haematology*, April 1983, vol. 53, no. 4, pages 563-573), applied to Claims 1 and 3-7; and
- (3) obviousness under 35 U.S.C. § 103(a) in view of Amiral (US 5,466,582) as evidenced by Thorpe *et al.* (US 6,312,694) in combination with Zuk *et al.* (US 4,281,061), applied to Claims 34-37.

VII. ARGUMENT

Regarding (1): Rejection of Claims 1-7 Under 35 U.S.C. § 102(b)

Claims 1-7 stand rejected under 35 U.S.C. § 102(b), as they are said to be anticipated by Amiral (US 5,466,582) as evidenced by Thorpe *et al.* (US 6,312,694).

Amiral describes a method to detect thrombocytopenia. In this method, a sample of patient plasma is mixed with a complex of heparin and platelet factor 4, and the resulting mixture is tested to determine the presence or absence of antibodies to the complex. The complex to be used in the method of Amiral can also be a heparin/platelet complex or a heparin/platelet factor 4/platelet complex, both of these complexes incorporating whole, intact platelets. Amiral does not describe a method using thrombospondin-1 -- isolated, synthetic or recombinant -- in any way.

The protein components of the complex of Claims 1-7 cannot be in platelets. Rather, the protein components of the complex of Claims 1-7 are isolated from platelets or they are made synthetically or by recombinant DNA methods.

In the section of the Amiral patent entitled "Aim of the Invention" (column 2, lines 36-50), it is stated:

“This novel technical solution is based on the detection, (i) by means of specific antigenic substance (Ag), (ii) of an antibody-type immunological material contained in the plasma of the subject to be tested and selected from the group consisting of anti(Y) antibodies (where Y is especially Z, or Z-Ag complexes) directed especially against the inductor drug Z and its complexes with Ag. In the light of the results of the work undertaken by the Applicant, it has been found that in warm-blooded animals, especially man and other mammals, the administration of a thrombopenia-inducing drug produces anti(Z), anti(Z-platelet), anti(Z-Ag) and anti(Z-Ag-platelet) antibodies leading to blood platelet aggregation or activation and causing thrombopenia.”

In US 5,466,582, Amiral described complexes as above in which Ag = platelet factor 4 and Z = heparin. However, Amiral described no complexes including isolated thrombospondin-1. Amiral did not incorporate thrombospondin-1 into any complexes with heparin or platelet factor 4. It should be noted that a publication by Amiral *et al.* (*Thrombosis and Haemostasis* 73(1):21-28, 1995; Reference AR of Information Disclosure Statement) states the following on page 23, first paragraph: “The specificity of the type II HIT [heparin-induced thrombocytopenia] antibodies for PF4 [platelet factor 4] was assessed as there was no antibody binding to the other heparin-binding proteins (i.e., ATIII, HCII, Vitronectin, Fibronectin, HRGP, β -Thromboglobulin, Thrombospondin, PDGF), in the presence or in the absence of heparin.” Thus, one of skill in the art at the time of the invention had no reason to believe that thrombospondin-1/heparin complexes or platelet factor 4/heparin/thrombospondin-1 complexes could form in humans or could be made as isolated complexes.

In the Office Action dated 30 December 2003, the Examiner pointed out several passages in Amiral that were said to disclose the isolated complexes of Claims 1-7. It is difficult to discern the meaning of what the Examiner has written.

At column 4, line 31, “Jean Amiral discloses an isolated complex that includes heparin binding proteins platelets.” The line referred to is a definition of the term *hep* to mean heparin, its derivatives or analogs, including their complexes with platelets. Any complex referred to here is between heparin and whole platelets. Such complexes do not include platelet factor 4 and thrombospondin-1 as described in Claims 1-7.

Column 5, lines 13-29 of Amiral, the Examiner interprets to say, “These platelets proteins are antigenic fractions that can induce anti-heparin antibodies.” The “antigenic substance” of column 5, lines 13-29 is not identified in the paragraph pointed out by the Examiner, but in column 6, lines 43-64, it is identified as platelet factor 4 and fractions containing platelet factor 4 but not containing thrombospondin-1.

The Examiner states, “The drug heparin is mixed with a complex of antigenic substances to determine the presence of antibodies (see summary).” The summary states, “According to the invention, a sample is mixed with a complex of an antigenic substance such as platelet factor 4 (pF4) and heparin to determine if the sample contains antibodies which react with the complex.” This does not describe the isolated complexes of Claims 1-7.

The Examiner states, “Heparin binding proteins were isolated from mammalian blood during clinical trials (col. 11, lines 10-35).” The lines of US 5,466,582 pointed out by the Examiner do not describe the isolation of heparin binding proteins from blood during clinical trials. Rather, the referenced lines describe a comparison of two methods to test for heparin-induced thrombocytopenia. The older method involves adding a sample of patient plasma to platelets in the presence of heparin and observing platelet aggregation. The newer method described by Amiral is described in Example 2 in column 10, and involves adding a sample of patient plasma to purified platelet factor 4 in the presence of heparin. In the case of the older method, the platelet proteins remain on the surface of the platelet, and are not isolated, recombinant, synthetic or chimeric, as are the proteins of Claims 1-7. In the case of the newer method, the only platelet protein used is isolated platelet factor 4. Thorpe *et al.* did not state, and gave no evidence, that thrombospondin-1 and platelet factor 4 are known to associate with heparin in a single ternary complex comprising heparin, platelet factor 4 and thrombospondin-1.

The Examiner continues, “Although Jean Amiral does not particularly point out what the platelet proteins are, evidence is provided by Thorpe *et al.* that thrombospondin-1 and platelet factor 4 are found in platelet alpha granules and are known to associate with heparin (See USP#6,312,5694, col. 99, lines 47-50).” It is difficult to determine which platelet proteins the Examiner is referring to in this sentence, however, the platelet proteins to be included in the assay of Amiral are described in column 6, lines 43-64, and they do not include any thrombospondin-1. Thorpe *et al.* report at column 99, lines 47-50 that each of the proteins

platelet factor 4 and thrombospondin-1 can bind to heparin, and that each of them is found in platelets.

The Examiner reasons, “Jean Amiral discloses that heparin bind to platelet complexes, therefore it is inherent that thrombospondin-1 and platelet factor 4 will be included in that complex.” Appellants do not claim a complex of heparin and whole platelets. Claims 1-7 place requirements on the proteins platelet factor 4 and thrombospondin-1 that are not met by the components of any complexes described by Amiral.

The Examiner seems to assume that Amiral necessarily produced a ternary complex of platelet factor 4, thrombospondin-1 and heparin. This is incorrect. Note column 6, line 43 through column 7, line 34 of Amiral, wherein the platelet factor 4 to be complexed with heparin is described. In all cases, the platelet factor 4 is recombinant, synthetic, complexed with proteoglycan, or in fractions containing proteins other than platelet factor 4, but in all cases the platelet factor 4 is purified away from thrombospondin-1. The preparation of platelet factor 4 described in Example 1 of Amiral (column 9, lines 26-65) completely separates platelet factor 4 from thrombospondin-1. Note that platelet factor 4 and thrombospondin-1 elute from a heparin agarose column in fractions well separated in ionic strength.

The Examiner also finds in the Amiral patent a description of an assay kit, stating in the Office Action of 30 December 2003, “Jean Amiral also discloses an assay kit to determine a heparin-induced thrombopenia (col. 10, lines 10-67). The platelet factor 4 and thrombospondin-1 are present at a ratio determined to be optimal for recognition especially since Thorpe et al. discloses that these heparin binding proteins associated with heparin.” The cited passage says nothing about thrombospondin-1. Fractions containing thrombospondin-1 are identified in Example 1 of Amiral, but are not included in any way in the kit.

The Examiner’s “Response to Arguments” in the Office Action of 30 December 2003 indicates that there may be some uncertainty as to the interpretation of *complex*. The term *complex* is not explicitly defined in the specification, but is used with the ordinary meaning as understood by one of ordinary skill in the art. The usual meaning of *complex* as used in the subject application, and as used in biology, is two or more chemical or biological entities joined by noncovalent chemical interaction (e.g., hydrophobic interactions of lipids; hydrogen and/or ionic bonding of an enzyme-substrate complex or an antigen-antibody complex). See, for

example, page 22, line 18 and page 23, line 29 in the specification. See also the use of *complex* in Amiral (US 5,466,582). See, for example, column 5, lines 25-26 (heparin/antigenic substance/platelet complexes) and column 6, line 55 (proteoglycan/platelet factor 4 complexes). No particular structural configuration is implied by *complex*.

The complexes made by Amiral (US 5,466,582) were of two components only: platelet factor 4 and heparin. See column 10, lines 25-28. The platelet factor 4 used in Example 2 was purified as in Example 1 and does not contain thrombospondin-1. Thrombospondin-1 was separated from platelet factor 4 in Example 1. See column 9, lines 47-50 and line 55. Claims 1-7 require three distinct chemical entities -- heparin, platelet factor 4 and thrombospondin-1 -- to be in one complex. The complex of Claims 1-7 is not described in Amiral.

Regarding (2): Rejection of Claims 1 and 3-7 Under 35 U.S.C. § 102(b)

Claims 1 and 3-7 stand rejected under 35 U.S.C. § 102(b) "as being anticipated by Gogstad *et al.* (*British Journal of Haematology*, April 1983, vol. 53, no. 4, pages 563-573)."

Gogstad *et al.* describe crossed immunoelectrophoresis of solubilized platelets or subfractions of platelets against rabbit antibodies prepared against whole human platelets. For the detection of heparin-binding proteins, the lower half of the intermediate gel contained heparin-Sepharose 4B (heparin covalently linked to Sepharose 4B). When heparin-Sepharose 4B was included in the intermediate gel for crossed electrophoresis of solubilized platelets, several immunoprecipitates observed in the absence of heparin-Sepharose 4B were missing or covered a reduced area, indicating that the antigens that were part of those immunoprecipitates were bound to the heparin-Sepharose 4B after the electrophoresis step performed in the second dimension. Platelet factor 4 bound to heparin-Sepharose 4B and thrombospondin-1 bound to heparin-Sepharose 4B as shown by the formation of separate immunoprecipitates observed in the absence of heparin-Sepharose 4B but not in the presence of heparin-Sepharose 4B.

Gogstad *et al.* (*British J. of Haematology* 53:563-573, 1983) shows that two different immunoprecipitates can form from (1) binding of platelet factor 4 to heparin-Sepharose 4B and (2) binding of thrombospondin-1 to heparin-Sepharose 4B. There was no demonstration that the three components of the complex of Claims 1 and 3-7 can bind together. Gogstad *et al.* do not teach a complex comprising heparin, platelet factor 4 and thrombospondin-1. There is no

evidence in Gogstad *et al.* that all three of these components can bind together in a ternary complex.

The Examiner stated that Gogstad *et al.* (*Br J Haematol*, 1983, 53: 563-573) teach a complex of platelet proteins that were isolated from alpha granules, and that these platelet proteins include platelet factor 4 and thrombospondin-1 bound to immobilized heparin. This is not true at all. Gogstad *et al.* studied the platelet proteins that interact with heparin-Sepharose 4B. In this work, Gogstad *et al.* solubilized platelet proteins in Triton X-100 and applied them to crossed immunoelectrophoresis against anti-platelet antibodies, using a medium in which an intermediate gel containing heparin covalently linked to Sepharose 4B was inserted. Gogstad *et al.* concluded that the platelets contain at least six heparin-binding proteins which are present on the platelet surface or capable of being exposed to the extracellular medium after the release-reaction (of proteins from the platelet surface) has occurred. These proteins are platelet factor 4, thrombospondin-1, GPIb and three proteins named in the Gogstad *et al.* paper as G4, 17 and 25.

The Gogstad *et al.* paper (*Br J Haematol*, 1983, 53: 563-573) does not discuss the possibility of a complex between heparin and any of the six mentioned proteins with which heparin can interact under some conditions (platelet factor 4, thrombospondin-1, GPIb, G4, 17 and 25). Gogstad *et al.* do not discuss anything about heparin, platelet factor 4 and thrombospondin-1 forming a ternary complex. Gogstad *et al.* teach that at least six platelet proteins can each interact individually with heparin under some conditions but not, as was said by the Examiner, that these proteins form a complex with heparin. There is no suggestion in Gogstad *et al.* that heparin can form a complex with more than one protein at any one time.

Appellants demonstrated that heparin (not the chemical entity heparin covalently linked with a support such as Sepharose 4B or agarose), thrombospondin-1 and platelet factor 4 interact together and form ternary complexes.

Regarding (3): Rejection of Claims 34-37 Under 35 U.S.C. § 103(a)

Claims 34-37 stand rejected under 35 U.S.C. § 103(a) "as being unpatentable over Amiral as evidenced by Thorpe *et al.* in view of Zuk *et al.* (US 4,281,061)."

The teachings of Amiral (US 5,466,582) and the teachings of Thorpe *et al.* (US 6,312,694) have been discussed above. Zuk *et al.* (US 4,286,061) teach an immunoassay method

and a kit in which predetermined amounts of the reagents for the immunoassay can be provided. Zuk *et al.* do not teach anything about the components of an assay for antibodies produced in heparin-induced thrombocytopenia patients, and do not mention heparin, platelet factor 4 or thrombospondin-1.

The Examiner states, “The teachings of Jean Amiral as evidenced by Thorpe *et al.* ... differ from the instant claims in not teaching all the components of a kit.” It is true that the teachings of Amiral as evidenced by Thorpe *et al.* do not include the components of the kits of Claims 34-37. Specifically, Amiral does not mention any of these: a buffered medium comprising isolated human thrombospondin-1 (Claims 34-36); a standardized positive control comprising known amounts of ternary complex reactive antibody (Claims 34-37); and a solid-phase support suitable for the immobilization of a platelet factor 4/heparin/thrombospondin-1 ternary complex (Claim 35). The teachings of Zuk *et al.* do not make up for the deficiencies of the Amiral patent, as the Zuk *et al.* patent also does not teach any of these components of the kits of Claims 34-37.

As discussed above, the Amiral patent does not teach that heparin, platelet factor 4 and thrombospondin-1 form a platelet factor 4/heparin/thrombospondin-1 ternary complex, nor does it teach the platelet factor 4/heparin/thrombospondin-1 complex as being the antigen recognized by antibodies produced in patients with heparin-induced thrombocytopenia. The Amiral patent teaches only a platelet factor 4/heparin binary complex.

One of ordinary skill in the art, studying the Amiral patent, might want to carry out an assay for antibodies that bind to platelet factor 4/heparin complexes, as platelet factor 4/heparin complexes are discussed by Amiral as the immunogen involved in heparin-induced thrombocytopenia. Combining the teachings of the Amiral patent with those of Zuk *et al.*, one of ordinary skill in the art might think of a kit containing reagents to produce platelet factor 4/heparin binary complexes and antibodies produced to platelet factor 4/heparin binary complexes. However, there is nothing in any of the references cited by the Examiner or in their combination that would lead one of ordinary skill in the art to put together a kit that will detect the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 complex, or a kit that would include thrombospondin-1 in any way. One of ordinary skill in the

art at the time of the invention would have had no reason to include thrombospondin-1 in isolated complexes to be used in diagnosing heparin-induced thrombocytopenia.

Respectfully submitted,

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CLAIMS APPENDIX

1. (Currently Amended) An isolated complex comprising heparin and the heparin binding proteins platelet factor 4 and thrombospondin-1 wherein each of the heparin binding proteins is an intact protein isolated from human platelets or produced using recombinant means, or a biologically active fragment prepared from a protein isolated from human platelets, or a recombinant protein, a variant recombinant protein, a synthetic peptide, or a chimeric protein.
2. (Currently Amended) The complex according to Claim 1 wherein the heparin binding proteins are isolated from human blood.
3. (Original) The complex according to Claim 1 wherein the platelet factor 4 is an isolated protein selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a recombinant human protein, a synthetic peptide, a recombinant human variant protein and a chimeric human protein.
4. (Original) The complex according to Claim 1 wherein the thrombospondin-1 is an isolated protein selected from the group consisting of: a protein isolated from human platelets, a fragment prepared from a protein isolated from human platelets, a

recombinant human protein, a synthetic peptide, a recombinant human variant protein and a chimeric human protein.

5. (Original) The isolated complex according to Claim 1 wherein the heparin platelet factor 4 and thrombospondin-1 are present at a ratio determined to be optimal for recognition by platelet factor 4/heparin/thrombospondin-1 ternary complex-reactive immunoglobulin present in a standardized positive control sample.
6. (Original) The isolated complex according to Claim 4 wherein the complex is preformed by combining 0.01-40 $\mu\text{g/ml}$ platelet factor 4, 0.01-1.0 U/ml unfractionated heparin and 0.01-40 $\mu\text{g/ml}$ thrombospondin-1.
7. (Original) The isolated complex according to Claim 5 wherein the complex is formed by combining 20 $\mu\text{g/ml}$ human platelet factor 4, 0.03 U/ml unfractionated heparin and 1 $\mu\text{g/ml}$ human thrombospondin.
34. (Original) A kit for an enzyme linked immunoadsorbent assay for detecting the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 antigen complex, comprising:
 - a buffered medium comprising heparin;
 - a buffered medium comprising isolated human PF4;
 - a buffered medium comprising isolated human TSP-1;

- a wash medium formulated to reduce nonspecific binding;
- at least one anti-human immunoglobulin reactive reagent detectably labeled with a reporter molecule, and having a specificity for at least one isotype of human immunoglobulin;
- a standardized positive control comprising known amounts of ternary complex reactive antibody;
- a negative control sample;
- a substrate for the reporter molecule; and
- a diluent reagent.
35. (Original) The kit according to Claim 34 further comprising a solid phase support suitable for the immobilization of a platelet factor 4/heparin/thrombospondin-1 ternary complex, wherein said solid phase support comprises a material selected from the group consisting of: polycarbonate, polyallomer, polypropylene, polyvinyl, nylon, nitrocellulose, polystyrene and maleic anhydride activated polystyrene.
36. (Original) A kit for detecting the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 antigen complex, comprising:
- a solid phase support material on which a platelet factor 4/heparin/thrombospondin-1 ternary complex has been immobilized; a buffered medium comprising isolated human TSP-1;
- a wash medium formulated to reduce nonspecific binding;

at least one anti-human immunoglobulin reactive reagent detectably labeled with a reporter molecule, and having a specificity for at least one isotype of human immunoglobulin;

a standardized positive control comprising known amounts of ternary complex reactive antibody;

a negative control sample; and

a diluent reagent.

37. (Previously Presented) A kit for detecting the presence of immunoglobulin reactive with a platelet factor 4/heparin/thrombospondin-1 antigen complex, comprising:
- a solid phase support material on which a platelet factor 4/heparin/thrombospondin-1 ternary complex has been immobilized;
- a wash medium formulated to reduce nonspecific binding;
- at least one anti-human immunoglobulin reactive reagent detectably labeled with a reporter molecule, and having a specificity for at least one isotype of human immunoglobulin;
- a standardized positive control comprising known amounts of ternary complex reactive antibody;
- a negative control sample; and
- a diluent reagent.